

COMPOSITIONS AND METHODS FOR NON-INVASIVE IMAGING OF SOLUBLE BETA-AMYLOID

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. Application Serial No. 10/431,202, filed May 7, 2003, currently pending and herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present disclosure relates to the detection of soluble beta-amyloid and the measurement of its local concentration in the brain of a subject without invasive procedures. The present disclosure also relates to novel compositions, imaging agents and benzofuran derivatives.

BACKGROUND OF THE INVENTION

[0003] The main histopathological characteristics of Alzheimer's disease ("AD") are the presence of neuritic plaques and tangles combined with associated inflammation in the brain. It is known that plaques are composed mainly of deposited (or insoluble in aqueous solution) fibrillar forms of the beta-amyloid ("A-beta") peptide. The formation of fully fibrillar aggregated A-beta peptide is a complex process that is initiated by the cleavage of the amyloid precursor protein ("APP"). After cleavage of APP, the monomeric form of A-beta can associate with other monomers, presumably through hydrophobic interactions and/or domain swapping, to form dimers, trimers and higher order oligomers. Oligomers of A-beta can further associate to form protofibrils and eventual fibrils, which is the main constituent of neuritic plaques. It has recently been shown that soluble oligomers (soluble in aqueous buffer) of A-beta may contribute significantly to neuronal dysfunction. In fact, animal models suggest that simply lowering the amount of soluble A-beta peptide, without affecting the levels of A-beta in plaques, may be sufficient to improve cognitive function.

[0004] Presently, the only definitive method of AD diagnosis is postmortem examination of brain for the presence of plaques and tangles. The antemortem diagnosis of AD is difficult, especially during the early stages, as AD symptoms are shared among a spectrum of other dementias. Currently, AD diagnosis is achieved using simple cognitive tests designed to test a patient's mental capacity such as, for

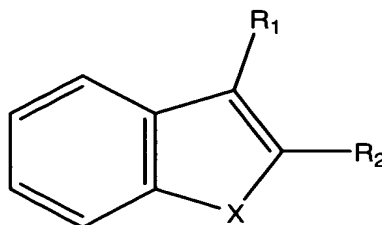
example, the ADAS-cog (Alzheimer's disease assessment scale - cognitive subscale) or MMSE (Mini-mental state examination). The subjective nature and inherent patient variability is a major shortcoming of diagnosing AD by such means. The fact that AD cannot be accurately diagnosed early creates a formidable challenge for pharmaceutical companies that aim to test anti-A-beta drugs as therapy to slow or halt AD pathogenesis. Furthermore, even if AD could be detected early and patients could be treated with A-beta lowering compounds, there is currently no way to know if the therapy is clinically efficacious. Therefore, a significant need exists to develop methods of measuring the soluble A-beta peptide levels locally in the brain.

[0005] Diagnosing AD by directly measuring levels of beta-amyloid noninvasively has been attempted by the targeted imaging of senile plaques. This approach fails as a specific measure of soluble A-beta peptide because current A-beta targeted imaging agents are directed at insoluble aggregates that are characteristic of A-beta fibrillar deposits in the brain. Further, targeted imaging of plaques may not provide early diagnosis, as large plaque burden is mostly associated with mid to late stage disease. Moreover, it has not been shown that current anti-A-beta therapies will affect fibrillar deposits appreciably to detect by imaging techniques at clinically relevant time points.

[0006] Alternatively, in vitro measures of A-beta may be specific for soluble A-beta in the cerebral spinal fluid, but lacks the necessary selectivity for local A-beta in the brain that is necessary for direct, accurate assessment of brain levels of soluble A-beta species. To date, the targeted non-invasive measurement and imaging of soluble A-beta peptide species (including monomer, dimers, trimers and n-oligomers) that exist in the central nervous system ("CNS") have not been addressed.

SUMMARY

[0007] This disclosure relates a compound having the following Formula I



wherein X is selected from a group that comprises at least one of oxygen, nitrogen and sulfur; R¹ is selected from the group consisting of substituted or unsubstituted alkyl hydroxy, amide, urea, and urethane; and R² is a hydrocarbon radical selected

from the group consisting of a C₁-C₃₂ substituted or unsubstituted branched or straight chain alkyl, cycloaliphatic, aryl and heteroaryl, including five membered rings, six member rings, and fused systems thereof.

[0008] In another aspect, an imaging agent is described, comprising the compound described in Formula I and a label.

[0009] In another aspect, methods of detecting at least one of A-beta species and amyloidogenic peptides comprising the steps of providing a sample suspected of comprising A-beta species or amyloidogenic peptides, applying an imaging agent comprising a compound described in Formula I, and detecting an amount of imaging agent bound to the at least one of A-beta species and amyloidogenic peptides.

[0010] In another aspect, methods of assessing an amyloid -related disease comprising the steps of administering to a subject an imaging agent comprising a compound as described in Formula I and detecting the imaging agent bound to at least one of A-beta species and amyloidogenic peptides.

[0011] In yet another aspect, methods of non-invasively assessing the therapeutic efficacy of therapies in a subject are described which include the steps of administering to a subject a first dose of a composition described in Formula I, and non-invasively obtaining a baseline measurement of the imaging agent within the subject, administering to the subject a therapy to be evaluated, administering to the subject a second dose of said composition, non-invasively obtaining a second measurement of the imaging agent within the subject, and comparing the two or more measurements separated in time, wherein an increase or decrease in the amount of the imaging agent present indicates the efficacy of the therapy.

DETAILED DESCRIPTION

[0012] The present disclosure relates to a method of non-invasively assessing levels of soluble A-beta to diagnose amyloid-related diseases, including Alzheimer's disease. This method qualitatively and quantitatively determines soluble A-beta levels in vivo. This method can also be used to determine the efficacy of related therapies used for amyloid-related diseases. To assess the soluble A-beta levels, a labeled diagnostic imaging agent is delivered to a subject. Typically, the subject is a mammal and can be human. The labeled imaging agent contains at least a chemical entity that binds to soluble A-beta and a chemical entity that emits a signal detectable by an imaging modality. The labeled imaging agent is delivered to a subject by a medically appropriate means. After allowing a clearance time according to the label chosen, the

amount of imaging agent bound to soluble A-beta is determined by noninvasively measuring the emitted signal using an imaging modality. The visual and quantitative analyses of the resulting images provide an accurate assessment of the global and local levels of soluble A-beta in the brain.

[0013] “A-beta species” herein refers to A-beta soluble monomers, soluble oligomers, and insoluble fibrils. “Amyloidogenic peptides” herein refer to peptides or proteins that have undergone or have the propensity to undergo an amyloidogenic process to form aggregates called amyloid, which have a secondary structure of cross-beta sheets, are birefringent under polarized light, stain with the histological stain Congo red, and are fibrillar in nature.

[0014] The present disclosure also relates to a method of labeling and detecting A-beta species and amyloidogenic peptides as well as quantitatively measuring the amount of A-beta species and amyloidogenic peptides in vitro, ex vivo, and in situ. The agent that binds to A-beta species and amyloidogenic peptides is detected by the agent’s emitted signal, such as emitted radiation, fluorescence emission, and optical properties of the agent. A-beta species and amyloidogenic peptides from at least cell culture, post-mortem human tissue, animal models of disease, and synthetic and recombinant sources are typically exposed to excess agent for a period of incubation. Agent that is nonspecifically bound or free in the incubation solution is blocked or washed away to leave agent specifically bound to A-beta species and amyloidogenic peptides that is detectable with common microscopic, widefield imaging, radiometric, fluorescent, optical, and analytical techniques. The detectable signal may be converted to numerical values to quantify the amount of targeted A-beta species and amyloidogenic peptides.

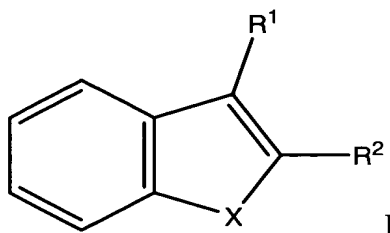
[0015] The chemical entity of the imaging agent that binds to soluble A-beta can bind to monomers, dimers, trimers and/or oligomers comprised of a larger number of A-beta peptides up to 24 A-beta peptides. More specifically, the soluble A-beta species to which the imaging agent can bind include monomers, dimers, trimers, and oligomers of A-beta 1-38, A-beta 1-39, A-beta 1-40, A-beta 1-41, A-beta 1-42, A-beta 1-43 or any combination thereof. The A-beta peptide in soluble monomer or oligomer forms can be derived ex vivo, by recombinant means, or synthetically. The soluble A-beta includes monomeric and low oligomeric A-beta that is soluble in an aqueous solution. In some embodiments, the soluble A-beta is of a type that remains in the supernatant of aqueous solution after centrifugation at 15000 times gravity. In some

embodiments, the soluble A-beta includes A-beta monomers and its aggregates that do not exhibit green birefringence when stained by Congo red.

[0016] The imaging agent that binds to soluble A-beta or otherwise reports on the presence of soluble A-beta can be derived from a natural source or be man made and be a small molecule, peptide, protein, enzyme, nucleic acid, nucleic acid sequence, dendrimer, polymer, antibody or antibody fragment.

[0017] The term "small molecule" means a molecule having a molecular weight of equal to or less than about 5000 daltons. In certain embodiments the small molecule has a molecular weight in the range of 300 to 2000 daltons. As well known in the art, such compounds may be found in compound libraries, combinatorial libraries, natural products libraries, and other similar sources, and may further be obtained by chemical modification of compounds found in those libraries, such as by a process of medicinal chemistry as understood by those skilled in the art, which can be used to produce compounds having desired pharmacological properties.

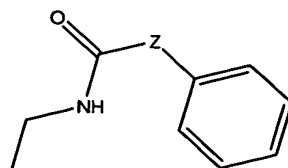
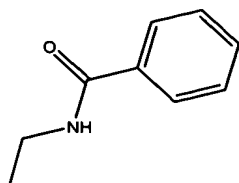
[0018] In one embodiment, certain compounds and their derivatives are useful as imaging agents that bind to soluble A-beta. The compositions described herein exhibit nanomolar affinity to soluble A-beta, as determined by fluorescence binding assays. Suitable compounds and derivatives include those represented by the following Formula I:



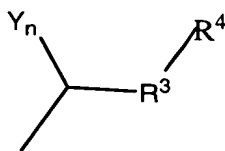
wherein X is selected from a group that comprises at least one of oxygen, nitrogen, or sulfur; R¹ is a hydrocarbon radical; and R² is a hydrocarbon radical or halogen. Preferably, R² is a hydrocarbon radical. By halogen is meant to include any of the entities known by those of ordinary skill in the art to be halogens, including, but not limited to bromine, fluorine, chlorine, and iodine. By hydrocarbon radical is meant any hydrocarbon radical, including substituted and unsubstituted, saturated or unsaturated, branched, straight chain alkyl, aryl, heteroaryl, cycloaliphatic radicals or any combination of the above. R¹ and R² may further be substituted with one or more heteroatom of oxygen, nitrogen, sulfur or halogen, such as chlorine, bromine, or

fluorine. In one embodiment, R^1 is C_1 - C_{10} alkyl hydroxy, an amide group, a urea group, or a urethane group and R^2 is a C_1 - C_{32} branched or straight chain alkyl, substituted or unsubstituted; a substituted or unsubstituted aryl group; a substituted or unsubstituted cycloaliphatic group. The term aryl and heteroaryl as used herein is intended to include, but not be limited to, five membered rings, six membered rings and fused ring systems thereof. The term "alkyl" as used in the various embodiments of the present invention is intended to designate both linear alkyl, branched alkyl, aralkyl, cycloalkyl, bicycloalkyl, tricycloalkyl and polycycloalkyl radicals containing carbon and hydrogen atoms, and optionally containing atoms in addition to carbon and hydrogen, for example atoms selected from Groups 15, 16 and 17 of the Periodic Table, including but not limited to oxygen, sulfur, nitrogen, fluorine, bromine and chlorine. The term "alkyl" also encompasses that alkyl portion of alkoxide groups. In various embodiments normal and branched alkyl radicals include as illustrative non-limiting examples C_1 - C_{32} alkyl optionally substituted with one or more groups selected from C_1 - C_{12} alkyl, C_3 - C_{15} cycloalkyl or aryl; and C_3 - C_{15} cycloalkyl optionally substituted with one or more groups selected from C_1 - C_{32} alkyl. Some particular illustrative examples comprise methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tertiary-butyl, pentyl, neopentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl. Some illustrative non-limiting examples of cycloalkyl and bicycloalkyl radicals include cyclobutyl, cyclopentyl, cyclohexyl, methylcyclohexyl, cycloheptyl, bicycloheptyl and adamantyl. In various embodiments aralkyl radicals are those containing from 7 to about 14 carbon atoms; these include, but are not limited to, benzyl, phenylbutyl, phenylpropyl, and phenylethyl. In various embodiments aryl radicals used in the various embodiments of the present invention are those substituted or unsubstituted aryl radicals or fused aromatic radicals containing from 6 to 18 carbon atoms. Some illustrative non-limiting examples of these aryl radicals include C_6 - C_{15} aryl optionally substituted with one or more groups selected from C_1 - C_{12} alkyl, C_3 - C_{15} cycloalkyl or aryl. Some particular illustrative examples of aryl radicals comprise substituted or unsubstituted phenyl, biphenyl, toluyl and naphthyl.

[0019] In yet another embodiment of the present invention, R^1 is alkyl hydroxyl, an amide group, a urea group or a urethane group such as those of the following formula

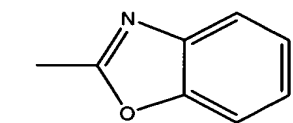


wherein Z is NH or S, or R^1 has the following structure

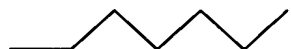


wherein Y is H or O, n is equal to the integer 1 or 2, R^3 is O or NH and R^4 is an acyl, substituted or unsubstituted aryl, ureas or urethanes. Further, nonlimiting examples of R^2 include those shown in Table 1 below.

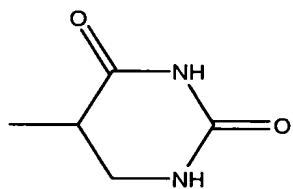
Table 1



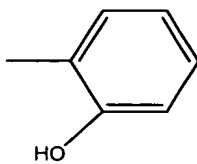
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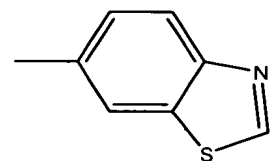
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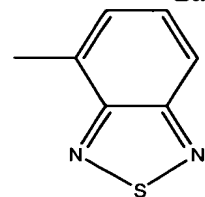
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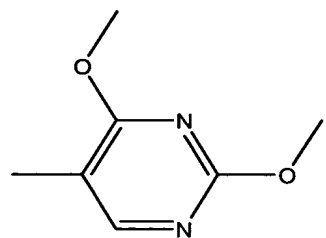
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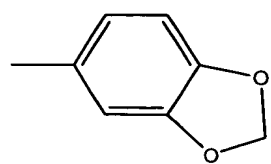
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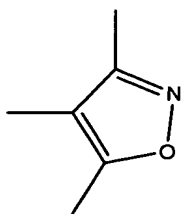
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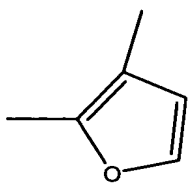
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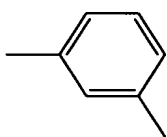
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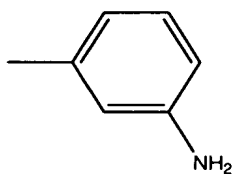
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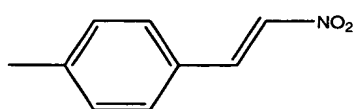
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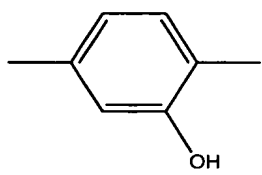
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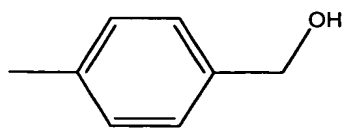
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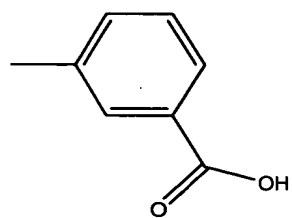
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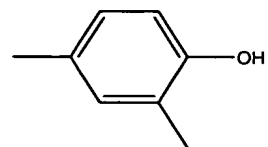
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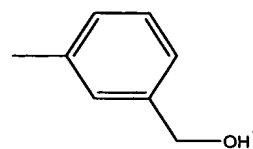
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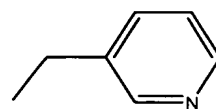
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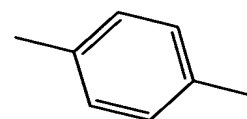
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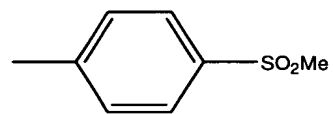
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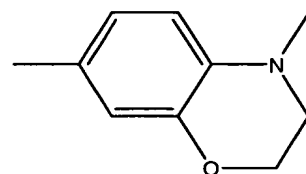
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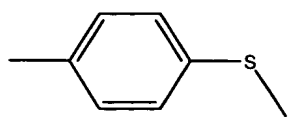
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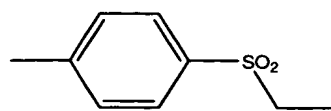
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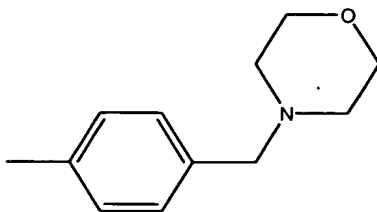
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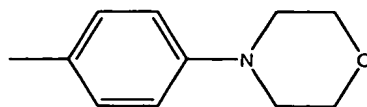
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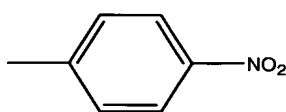
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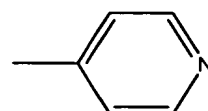
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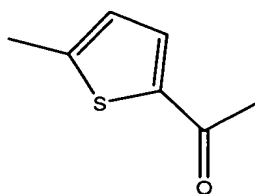
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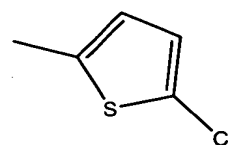
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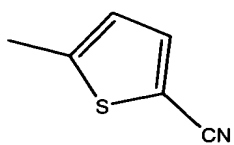
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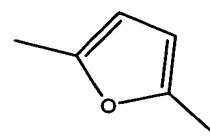
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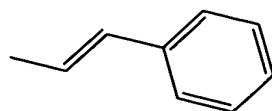
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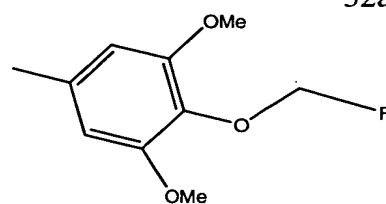
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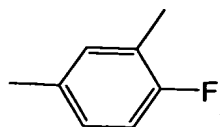
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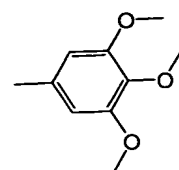
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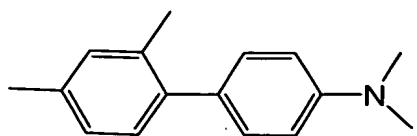
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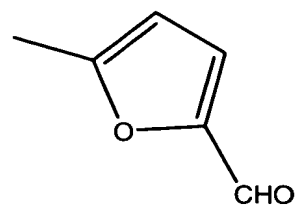
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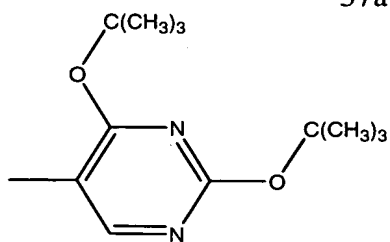
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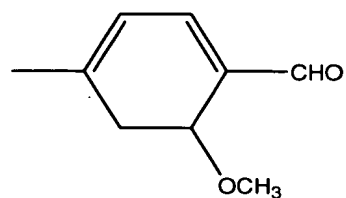
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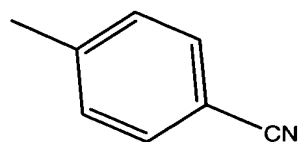
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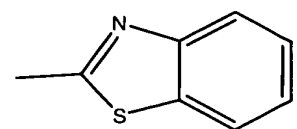
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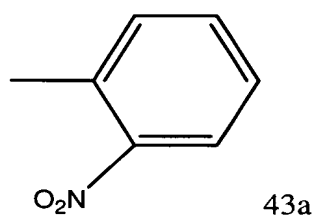
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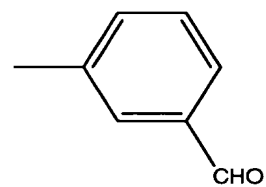
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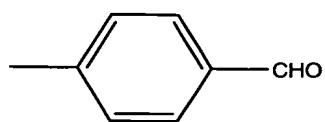
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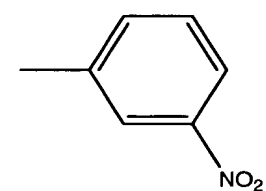
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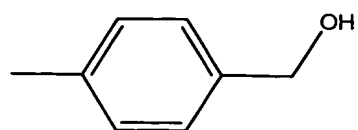
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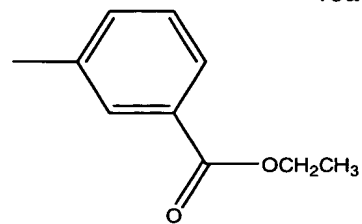
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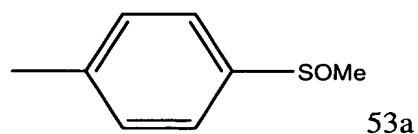
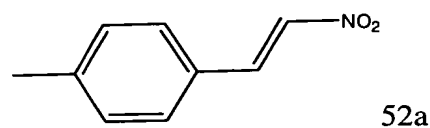
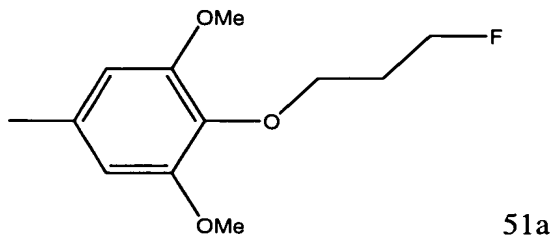
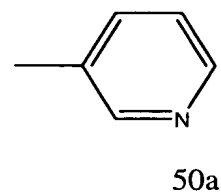
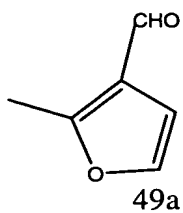
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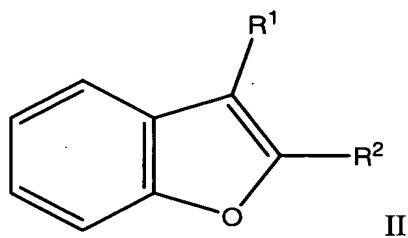
47a



48a

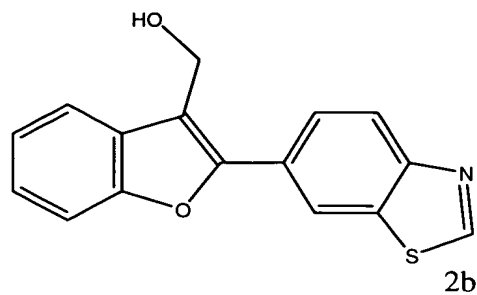
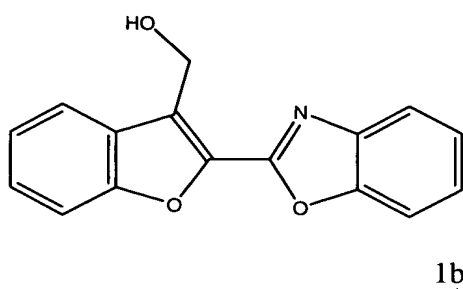


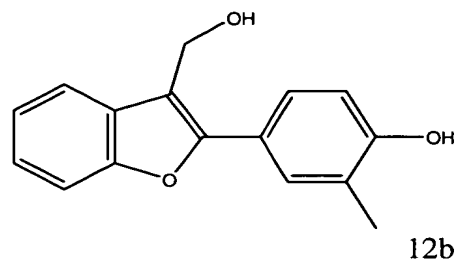
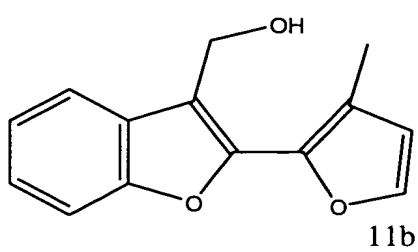
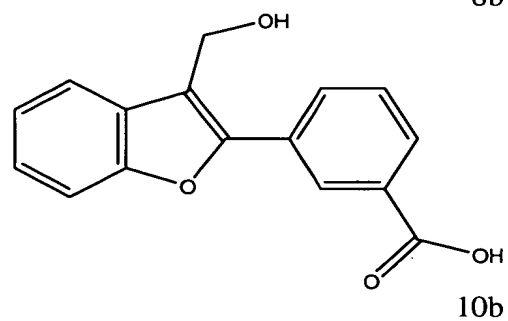
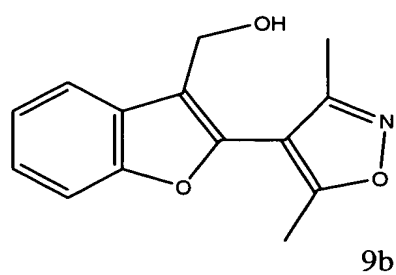
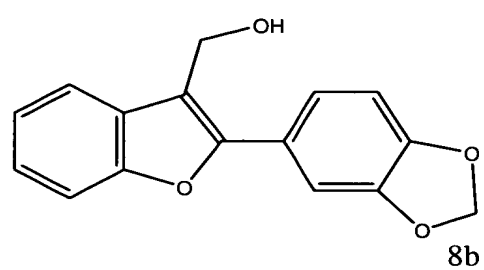
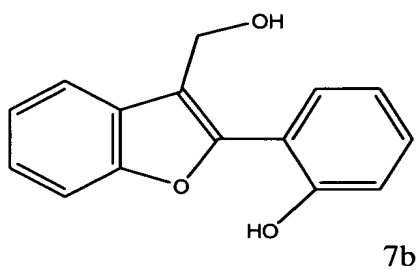
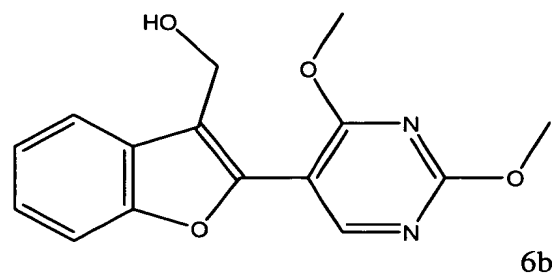
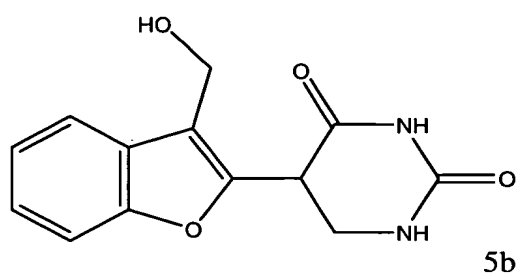
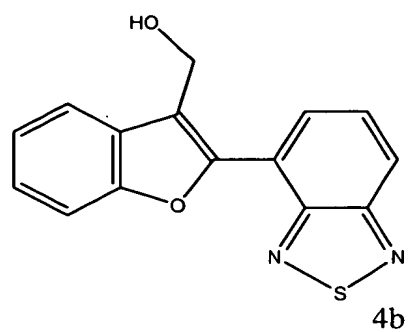
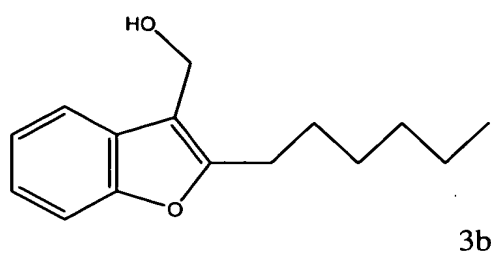
[0020] In yet another embodiment, useful imaging agents include benzofuran derivatives such as those of the following Formula II:

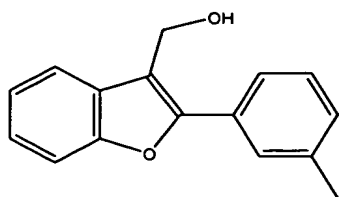


wherein R^1 and R^2 are as described above. More particularly, certain benzofuran derivatives having a formula as described in Table 2 are useful as imaging agents for A-beta.

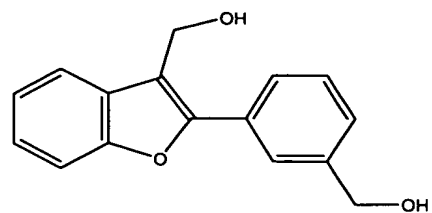
Table 2



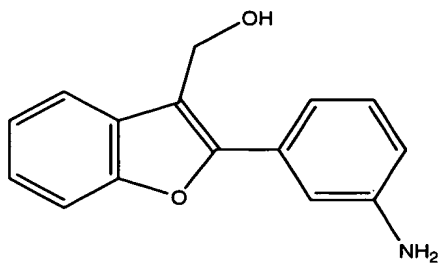




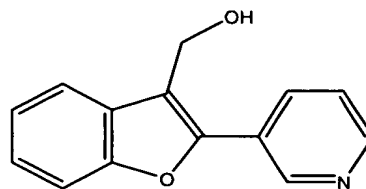
13b



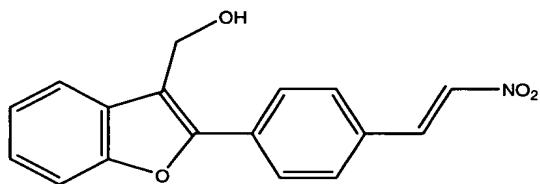
14b



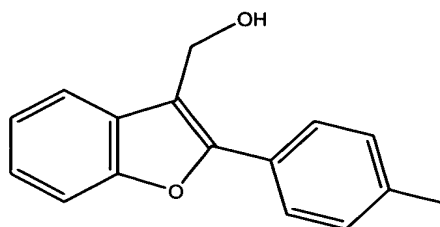
15b



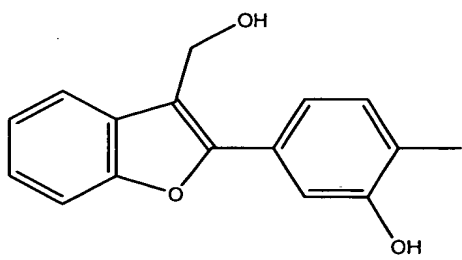
16b



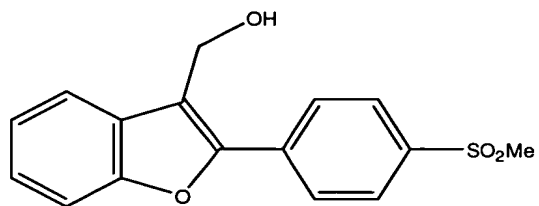
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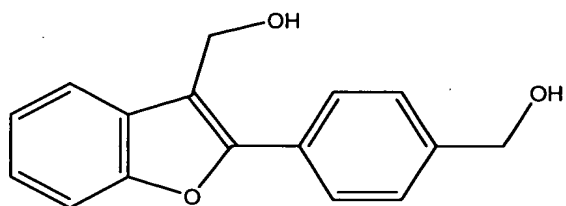
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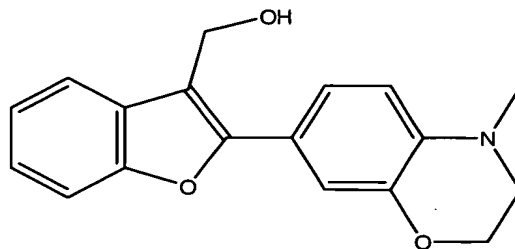
19b



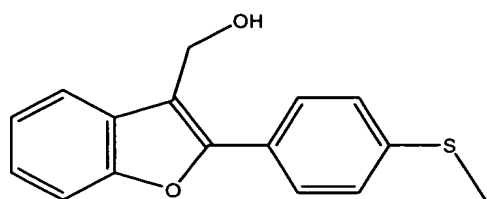
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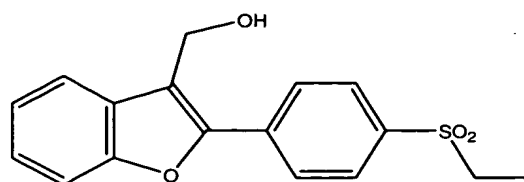
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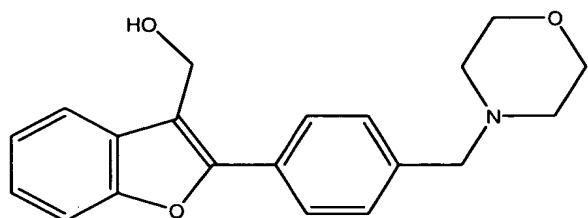
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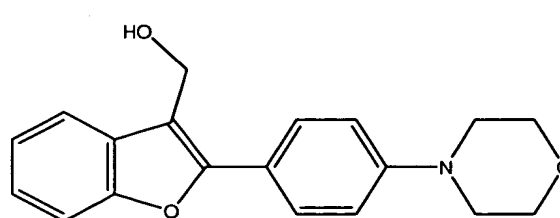
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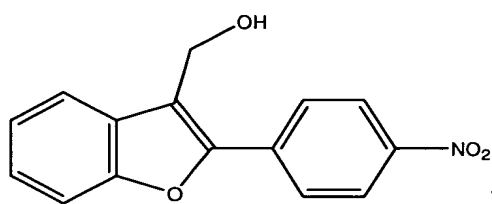
24b



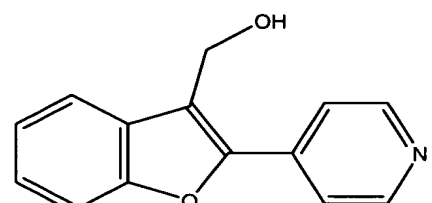
25b



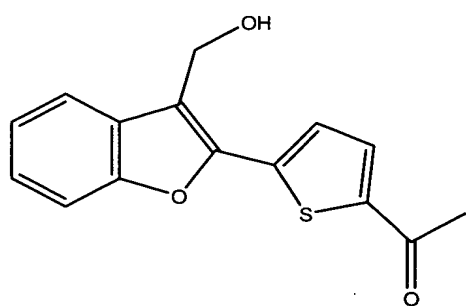
26b



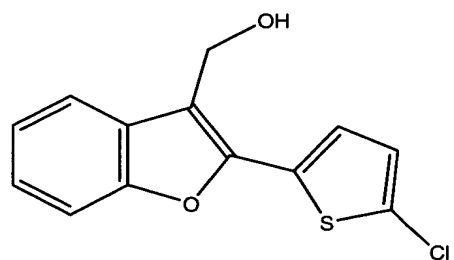
27b



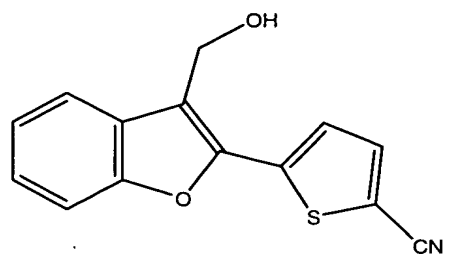
28b



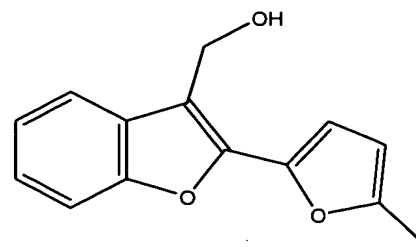
29b



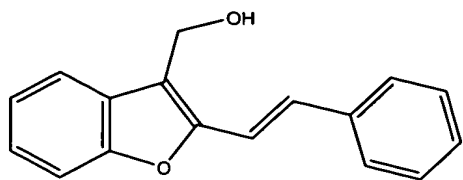
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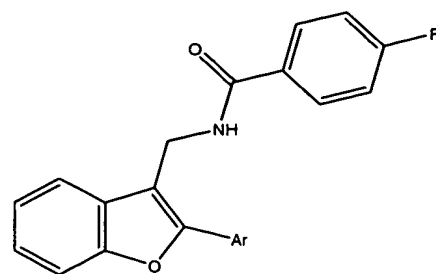
31b



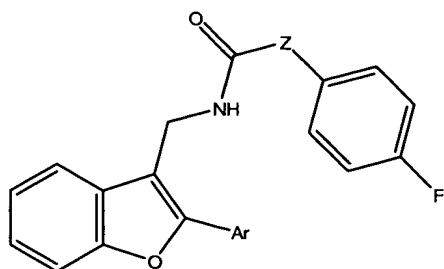
32b



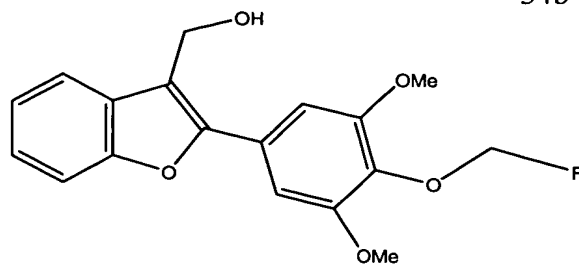
33b



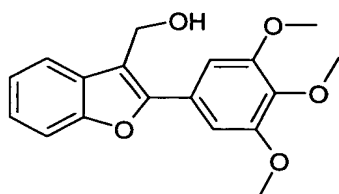
34b



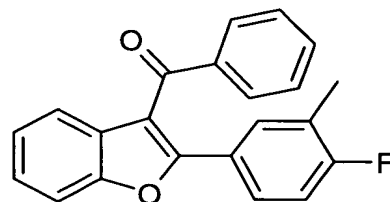
35b



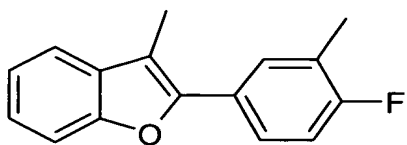
36b



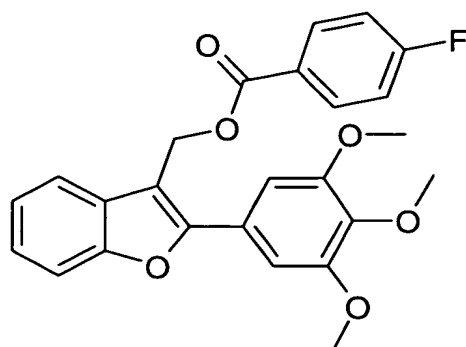
37b



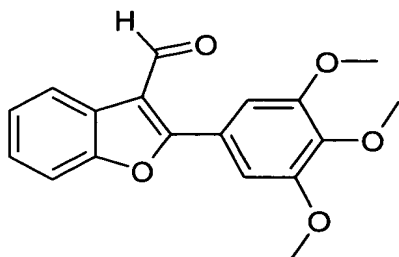
38b



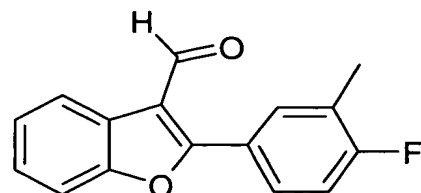
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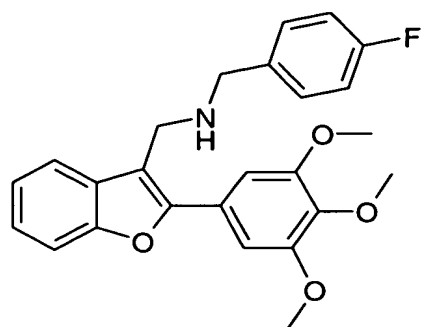
40b



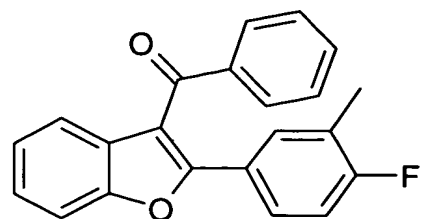
41b



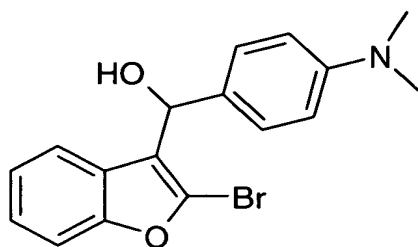
42b



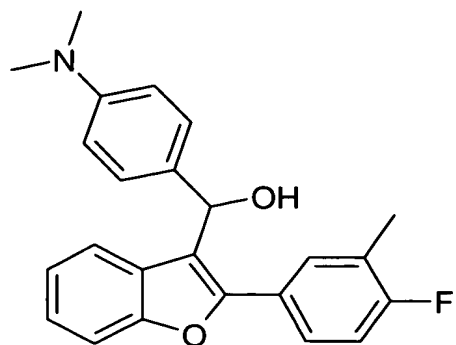
43b



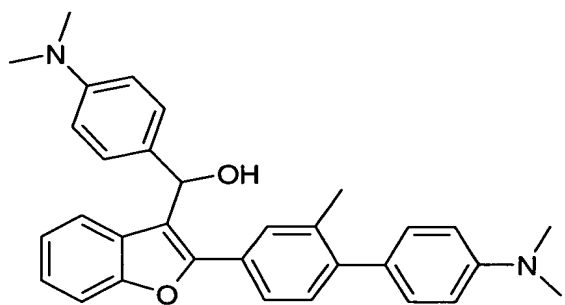
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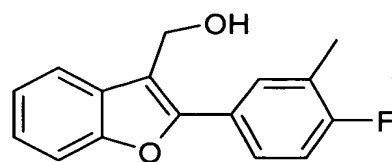
45b



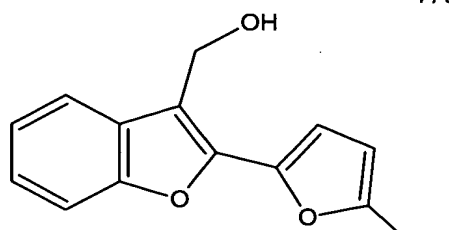
46b



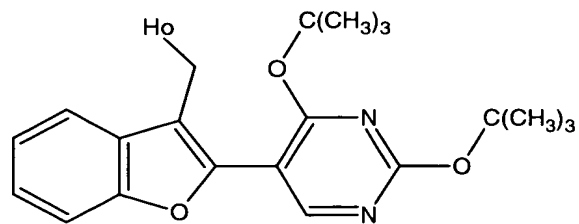
47b



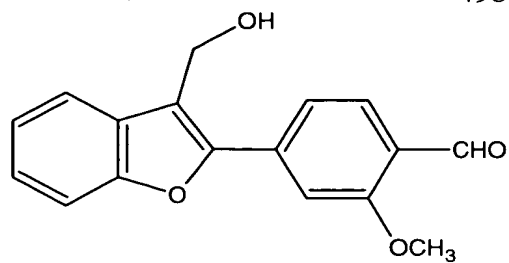
48b



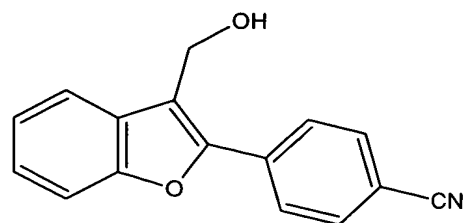
49b



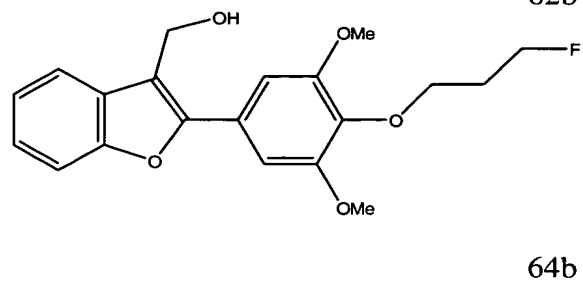
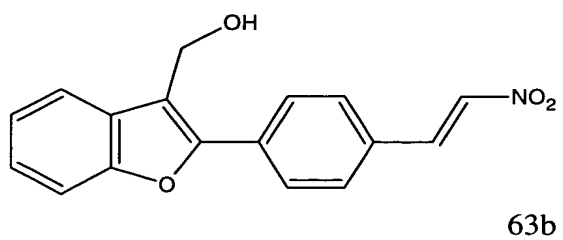
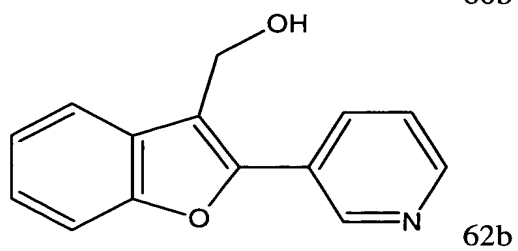
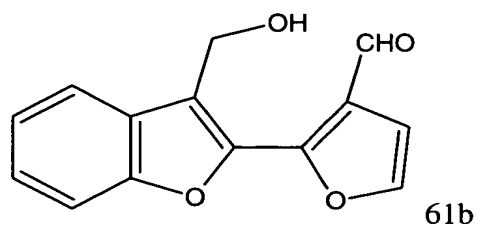
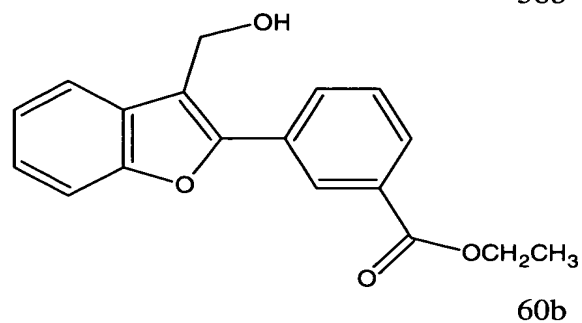
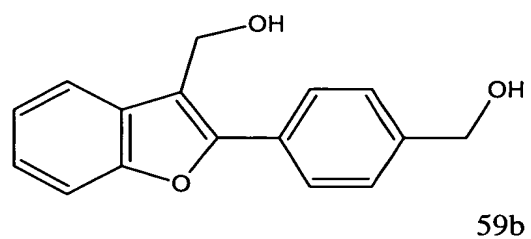
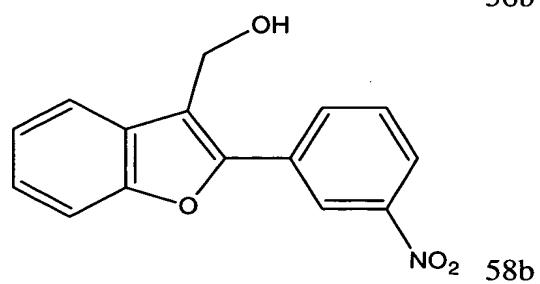
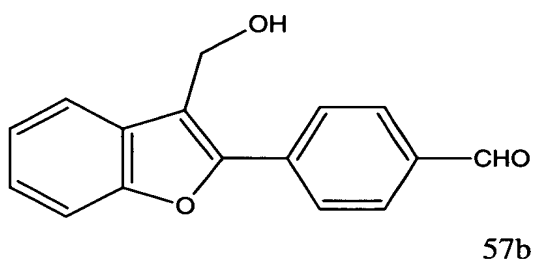
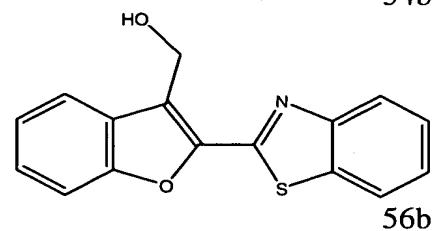
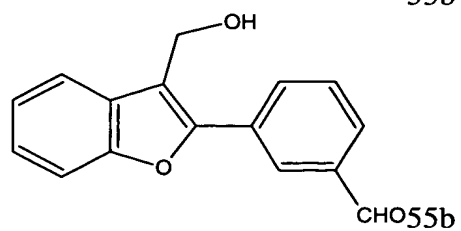
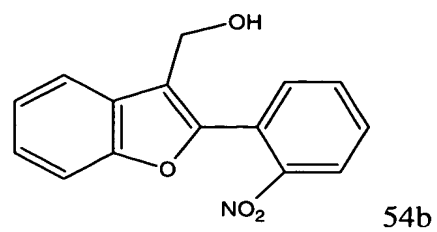
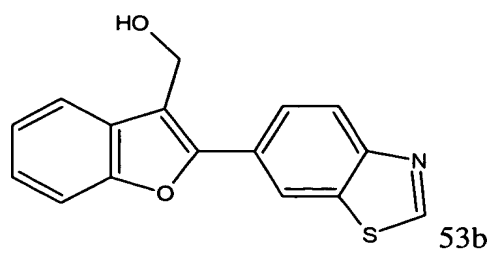
50b

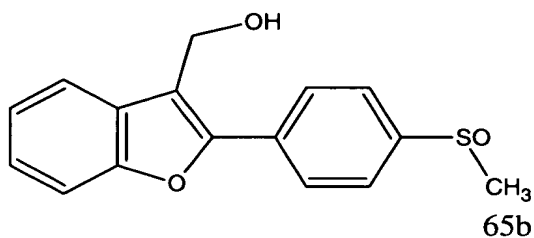


51b



52b





wherein Z is NH, O, S; Ar is substituted phenyl, pyridinyl, thiophenyl, furanyl, and the like, as in the examples above. As is known in the art, “Et” is ethyl and “Me” is methyl.

[0021] Substituted 2-phenyl-3-alkyl benzofurans similar to those shown in Formula II have been synthesized by the Lewis acid – mediated condensation of benzyl with phenols and aryl ethers, Palladium – catalyzed cross-coupling of benzyl ketones and α,β -unsaturated carbonyl and phenolic compounds with o-dibromobenzenes, and McMurry-type reductive cyclization of dicarbonyl compounds catalyzed by low-valent Ti. Also, 2,3-diphenyl benzofurans have been prepared by the cyclodehydration of α -aryloxydeoxybenzoins catalyzed by phosphoric acid. 2-Aryl-3-allylbenzofurans have been synthesized by the Pd -catalyzed cyclization of 2-alkynylphenols with allyl carbonates. Pd-catalyzed cyclizations are also the preferred method for the synthesis of a variety of 2-alkylbenzofurans and 2-arylbenzofurans.

[0022] These methods, however, are not versatile enough to allow for the synthesis of a large variety of benzofurans of the general structure shown. A more versatile and general approach is depicted below. Using the 2-bromo-3-formylbenzofuran as a starting point, reaction with commercially available organozinc reagents leads to the introduction of functionalized moieties at the 3-position. Suzuki coupling using the 2-bromo-functionality allows for the introduction of functionalized 2-arylsubstituents, in the presence of a variety of functional groups. These two steps could be interchanged, if practical advantages arise. The use of microwave accelerated organic synthesis allows for the rapid evaluation of synthetic strategies and optimization of reaction conditions before molecular diversity through high throughput organic synthesis is pursued. The formyl substituent permits an efficient entry to functionalized amine moieties via a reductive amination sequence.

[0023] Unlike the presently described imaging agents that bind to soluble A-beta, there are imaging agents and dyes that bind exclusively to insoluble deposits of A-beta or senile plaques. Small molecules that specifically bind to insoluble A-beta

deposits include, for example, small molecular weight molecules, such as Congo red, Chrysamine G, methoxy-X04, TZDM, [^{11}C]6, IMSB, Thioflavin(e) S and T, TZDM, 1-BTA, benzathiozole derivatives, [^{125}I]3, BSB, IMSB, styrylbenzene-derivatives, IBOX, benzoxazole derivatives, IMPY, pyridine derivatives, DDNP, FDDNP, FENE, dialkylaminonaphthyl derivatives, benzofuran derivatives, and derivatives thereof (see, e.g., U.S. Pat. Nos. 6,133,259; 6,168,776; 6,114,175).

[0024] Nucleic acid sequences and derivatives thereof have been shown to bind to insoluble senile plaques of A-beta, including mRNA for furin and amyloid precursor protein ("APP").

[0025] Peptides also have been developed as imaging agents for insoluble deposits of A-beta and senile plaques. The sequence specific peptides that have been labeled for the purpose of imaging insoluble A-beta includes the labeled A-beta peptide itself, putrescine-gadolinium-A-beta peptide, radiolabeled A-beta, [^{111}In]A-beta, [^{125}I]A-beta, A-beta labeled with gamma emitting radioisotopes, A-beta-DTPA derivatives, radiolabeled putrescine, KVLFF-based ligands and derivatives thereof.

[0026] Inhibitors of aggregated A-beta have been suggested to disrupt the formation of these aggregates by interacting with soluble and/or insoluble fibrils of A-beta. Examples of inhibitors or anti-aggregation agents include peptides of A-beta, KVLFF-based ligands, small molecular weight compounds, carbon nanostructures, rifamycin, IDOX, acridone, benzofuran, apomorphine, and derivatives thereof.

[0027] Agents have also been known to promote aggregation – agents such as A-beta₄₂, proteins, metals, small molecular weight compounds, and lipids. Agents that either promote aggregation or disaggregation of A-beta fibrils presumably interact with either soluble or insoluble A-beta or both, suggesting that developing compounds that exclusively bind A-beta is feasible.

[0028] Antibodies for A-beta are similar to KLVFF-derivative as they also interact with soluble and insoluble A-beta. Antibodies specific for soluble and insoluble A-beta can be prepared against a suitable antigen or hapten comprising the desired target epitope, such as the junction region consisting of amino acid residues 13-26 and/or the carboxy terminus consisting of amino acid residues 33-42 of A-beta. One suitable antibody to soluble A-beta is disclosed in Kaye, et al., Science, vol. 300, page 486, April 18, 2003. Synthetic peptides can also be prepared by conventional solid phase techniques, coupled to a suitable immunogen, and used to prepare antisera or monoclonal antibodies by conventional techniques. Suitable peptide haptens typically

will comprise at least five contiguous residues within A-beta and can include more than six residues. Synthetic polypeptide haptens can be produced by the Merrifield solid-phase synthesis technique in which amino acids are sequentially added to a growing chain (Merrifield (1963) J. Am. Chem. Soc. 85:2149-2156). Suitable antibodies include, for example, those of US Pat. Nos. 5,811,310; 5,750,349; and 5,231,000, R1282, 21F12, 3D6, FCA3542, and monoclonal and polyclonal antibodies for A-beta 1-40, 1-42 and other isoforms. Certain imaging agents have been developed that can report on the specific presence of a target molecule without binding to that molecule. In such instances the imaging agents are considered "activatable" because their signal is activated or unactivated based on the presence of a specific target molecule. Examples of such agents have been used for MRI and optical imaging (Li WH, Parigi G, Fragai M, Luchinat C, Meade TJ, Inorg Chem 2002 Jul 29;41(15):4018-24)(Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. Nat Biotechnol 2000 Mar;18(3):321-5) (Weissleder R, Tung CH, Mahmood U, Bogdanov A Jr Nat Biotechnol 1999 Apr;17(4):375-8).

[0029] The agents described above target either soluble or insoluble (i.e., fibrillar) A-beta. Agents may bind other proteins that aggregate to form amyloid in the central nervous system or peripherally in the body of a mammal. Diseases with amyloidogenic peptides may be called amyloidoses. Amyloidosis affecting the body other than the CNS are referred to as systemic amyloidosis whereas amyloidoses in the CNS include alpha-synuclein protein in Parkinson's disease, multiple myeloma-associated protein in amyloidomas, transthyretin in oculoneurohypophyseal A and Meningocerebrovascular A, A-beta peptide in Down's syndrome and hereditary cerebral hemorrhage with amyloidosis (HCHWA)-Dutch type, cystatin C in HCHWA-Icelandic type, and prion protein in transmissible spongiform encephalopathy.

[0030] The chemical entity of an imaging agent, including those described in Formulas I and II, that emits a detectable signal (also called a label) can be either inherent or a radiolabel, a paramagnetic label, an optical label and the like. "Label" is used hereinafter to include both inherent labels, such as the agents' own fluorescence or chemiluminescence, or a label added to the agent. The type of imaging modality available will be an important factor in the selection of the label used for an individual subject. For example, a radiolabel must have a type of decay that is detectable by the

available imaging modality. Suitable radioisotopes are well known to those skilled in the art and include beta-emitters, gamma-emitters, positron-emitters, and x-ray emitters. Suitable radioisotopes include ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{123}I , ^{125}I , ^{131}I , ^{51}Cr , ^{36}Cl , ^{57}Co , ^{59}Fe , ^{75}Se and ^{152}Eu . Isotopes of halogens (such as chlorine, fluorine, bromine and iodine), and metals including technetium, yttrium, rhenium and indium are also useful labels. Typical examples of metallic ions which can be bound are $^{99\text{m}}\text{Tc}$, ^{123}I , ^{111}In , ^{131}I , ^{97}Ru , ^{67}Cu , ^{67}Ga , ^{125}I , ^{68}Ga , ^{72}As , ^{89}Zr , and ^{201}Tl . For use with the present disclosure, radiolabels can be prepared using standard radiolabeling procedures well known to those skilled in the art. Preferably, the labeling will be accomplished by incorporation of one of the above-listed labels into one or both of the R^1 or R^2 groups.

[0031] The disclosed compounds can be radiolabeled either directly by incorporating the radiolabel directly into the compounds or indirectly by incorporating the radiolabel into the compounds through a chelating agent, where the chelating agent has been incorporated into the compounds. Such radiolabeling should also be reasonably stable, both chemically and metabolically, applying recognized standards in the art. Also, although the label can be incorporated in a variety of fashions with a variety of different radioisotopes, such radiolabeling should be carried out in a manner such that the high binding affinity and specificity of the unlabeled binding moiety is not significantly affected.

[0032] Preferred radioisotopes for in vivo diagnostic imaging by positron emission tomography ("PET") are ^{11}C , ^{18}F , ^{123}I , and ^{125}I , with ^{18}F being the most preferred. Typically, the labeled atom is introduced to the labeled compounds at a late stage of the synthesis. This allows for maximum radiochemical yields, and reduces the handling time of radioactive materials. When dealing with short half-life isotopes, an important consideration is the time required to conduct synthetic procedures, and purification methods. Protocols for the synthesis of radiolabeled compounds are described in Tubis and Wolf, Eds., "Radiopharmacy", Wiley- Interscience, New York (1976); Wolf, Christman, Fowler, Lambrecht, "Synthesis of Radiopharmaceuticals and Labeled Compounds Using Short-Lived Isotopes", in Radiopharmaceuticals and Labeled Compounds, Vol. 1, p. 345-381 (1973).

[0033] Paramagnetic labels can be metal ions are present in the form of metal complexes or metal oxide particles. Suitable paramagnetic isotopes include ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr , and ^{56}Fe . The paramagnetic label can be attached to the binding

moiety by several approaches. One approach is direct attachment of one or more metal chelators to the binding moiety of the imaging agent. Alternatively, the binding portion of the imaging agent can be attached to a paramagnetic metal ion or heavy atom containing solid particle, or to an echogenic gas microbubble. A number of methods can be used to attach imaging agent, which specifically binds to soluble A-beta, to paramagnetic metal ion or heavy atom containing solid particles by one of skill in the art of the surface modification of solid particles. In general, the imaging agent is attached to a coupling group that reacts with a constituent of the surface of the solid particle. The coupling groups can be any of a number of silanes, and also include polyphosphonates, polycarboxylates, polyphosphates or mixtures thereof, which react with surface hydroxyl groups on the solid particle surface, as described, for example, in U.S. patent application publication 2002/0159947 and which can couple with the surface of the solid particles, as described in U.S. Pat. No. 5,520,904.

[0034] The imaging agent itself can be fluorescent or can be tagged with optical labels that are fluorophores, such as fluorescein, rhodamine, Texas Red, and derivatives thereof and the like. The labels can be chemiluminescent, such as green fluorescent protein, luciferin, dioxetane, and the like. These fluorophore probes are commercially available, e.g., from Molecular Probes, Inc., Eugene, Oregon. The imaging agent that binds to soluble A-beta can be linked to the portion of the compound that emits a detectable signal by techniques known to those skilled in the art.

[0035] The labeled imaging agent can typically be administered to a patient in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the labeled A-beta binding compound to the patient, including sterile water, alcohol, fats, waxes, proteins, and inert solids may be included in the carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersing agent) can also be incorporated into the pharmaceutical composition. Carriers can contain a solution of the imaging agent or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous sterile carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine, 25% human serum albumin and the like. The solutions are preferably pyrogen-free, sterile, and generally free of particulate matter. The compositions can contain additional pharmaceutically acceptable substances as necessary to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting

agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate. The concentration of imaging agent in the composition solutions may vary as required. Typically, the concentration will be in trace amounts to as much as 5% by weight depending on the imaging modality and are selected primarily based on fluid volumes, and viscosities in accordance with the particular mode of administration selected. Preferably, the concentration is between about 0.1 and about 1 nmol, more preferably from about 0.1 nmol to about 0.5 nmol. The imaging agent may be present in several ml of injectable solution, as would be determined based on dose, and easily calculated by one of ordinary skill in the art. If the agent is labeled with ^{18}F , approximately 105 pmol of ^{18}F yields 10 mCi of a radiation dose initially. This amount of radioactivity is typical and considered safe in the current medical imaging procedures. A typical composition for intravenous infusion can be made to contain 250 ml of sterile Ringer's solution and up to 100 mg, preferably around 10 mg, of the imaging agent. The composition containing the imaging agent can be combined with a pharmaceutical composition and can be administered subcutaneously, intramuscularly or intravenously to patients suffering from, or at risk of, amyloid-related conditions.

[0036] The imaging agent is administered to a subject to determine the presence and amount of soluble amyloid in the subject. After administration, clearance time can, if desired, be permitted which allows the imaging agent to travel throughout the subject's body and bind to any available soluble A-beta whereas the unbound imaging agent passes through the subject's body. In a case where the imaging agent does not directly bind, but rather reports on the presence of the A-beta, sufficient time is allowed for a specific interaction to occur in which the reporter molecule is "activated". The clearance time will vary depending on the label chosen for use and can range from 1 minute to 24 hours. The imaging agent is then detected noninvasively in the subject's body by an imaging modality. The imaging modality can include positron emission tomography ("PET"), optical, single photon emission computed tomography ("SPECT"), magnetic resonance imaging ("MRI"), ultrasound, computed tomography ("CT"), and the like, depending on the label used, the modality available to medical personnel and the medical needs of the subject. Equipment and methods for the foregoing imaging modulations are known to those skilled in the art.

[0037] The imaging agent can be delivered and the imaging taken to determine the amount of soluble A-beta present in the subject's body as an indication of disease or

pre-disease states. The levels of soluble A-beta can be indicative of pre-disease conditions and therapies toward removal of the soluble A-beta and/or its precursors can prevent or forestall the onset of an amyloid-related disease, such as Alzheimer's disease. The removal of soluble A-beta can also improve the condition of a subject that already exhibits clinical signs of disease.

[0038] In another aspect, the present methods can be used to determine the efficacy of therapies used in a subject. By using multiple images over time, the levels of A-beta can be tracked for changes in amount and location. This method can aid physicians in determining the amount and frequency of therapy needed by an individual subject. In this embodiment, an imaging agent in accordance with the present disclosure is administered and a baseline image is obtained. The therapy to be evaluated is administered to the subject either before or after a baseline images are obtained. After a pre-determined period of time, a second administration of an imaging agent in accordance with their disclosure is given. A second or more images are obtained. By qualitatively and quantitatively comparing the baseline and the second image, the effectiveness of the therapy being evaluated can be determined based on a decrease or increase of the signal intensity of the second image or additional images.

[0039] The following nonlimiting Examples are shown and describe various embodiments of the present invention.

EXAMPLE 1

[0040] Preparation of 2-bromo-3-bromomethyl benzo[b]furan: To a suspension of N-bromosuccinimide (10.8g, 60 mmol) in 3-methylbenzofuran(4g, 30.26 mmol) and carbon tetrachloride (20 ml), was added benzoyl peroxide (100 mg) and the mixture was refluxed for 3 hrs. Fresh NBS was added (1.1 g) and the mixture was refluxed for 1 hr. Following the addition of more NBS (1g) and refluxing for one more hour, GC-MS analysis of the reaction mixture indicated that the desired product was accompanied by 10% mono-brominated and 8% tri-brominated products. The succinimide was filtered off, washed with CCl₄ until white and the solvent was stripped off to give a brown, viscous oil. Absolute ethanol (12 ml) was added, the solution was cooled to -25°C and the mass of yellow crystals was filtered at -25°C with a fritted canula. The crystals were washed at -40°C with 12 ml ethanol and dried

under vacuum to give the desired product as a mass of needle like crystals (95% pure by GC-MS), 7.672g (87.4%).

EXAMPLE 2

[0041] Preparation of 2-bromo-3-hydroxymethyl benzo[b]furan (2): To a 250 ml flask was added the dibromide (7.672g, 26.45 mmol) in dioxane (30 ml), followed by a solution of NaHCO₃ in water (30 ml). The mixture was heated to reflux for one hour, while stirring it vigorously. The cooled mixture was then diluted with water (150 ml) and extracted with dichloromethane (5x). The extract was washed with brine, dried, the solvent was stripped, and the resulting orange oil was dissolved in 12 ml chloroform and cooled to -20°C. The resulting crystals were filtered as before at -40°C, washed with cold chloroform and dried under reduced pressure to give the desired product as light yellow crystals (GC-MS purity 98%), 3.72g (62.2%).

EXAMPLE 3

[0042] Preparation of 2-bromo-3-formyl benzo[b]furan (3): To a 50 ml round bottom flask were added powdered pyridinium chlorochromate (443 mg, 2.055 mmol), Celite[®] (450 mg) and dry dichloromethane (20 ml). A solution of the alcohol 2 as in Formula I, wherein R¹=CH₂OH, R²=Br, X=O (226 mg, 1 mmol) in dichloromethane (1ml) was added and the mixture was stirred at r.t. in the dark for one hour. TLC (hexanes/ethyl acetate 4/1 v/v) indicated complete conversion (R_f 0.7 vs. R_f 0.3 for starting material). The mixture was diluted with ether, flushed through a 1 in plug of silicagel, the solvent was stripped and the residue was again flushed through a 1 in plug of silicagel, using hexanes/ethyl acetate 2/1 v/v. to give the desired product as light orange crystals (205 mg, 91%).

EXAMPLE 4

[0043] General procedure for the Suzuki coupling reaction: To a microwavable vial were added the 2-bromo-3-substituted benzofuran (0.1 mmol), arylboronic acid (2 equivalents), tris(dibenzylidene acetone) dipalladium (Pd₂dba₃, 0.03 equivalents), K₂CO₃ (1.5 equivalents) and degassed dimethylformamide (1 ml). The vial was purged with N₂, capped, and irradiated for 10 minutes at 120C (initial power 50W). Water was then added (2 ml), the mixture was extracted with ether (5x), washed with water, brine, dried over Na₂SO₄ and the crude product was purified by MPLC using

hexanes/ethyl acetate gradient. All reactions were analyzed for conversion prior to work up. Unless otherwise stated, cooling was not turned on during the reaction. Also, 10 minutes reaction time were generally found to result in complete consumption of the bromofuran starting material.

EXAMPLE 5

[0044] General procedure for the addition of organometallic reagents to aldehyde 3, as in Example 3, (Formula I, $R^1=CHO$, $R^2=Br$, $X=O$): To a dry 5 ml flask was added the aldehyde (0.2 – 1 mmol), dry ether (1.5 ml) and the mixture was cooled to 0C. The organometallic reagent solution (1.25 equivalents) was then added dropwise and the mixture was stirred at 0C for 30 minutes. At this time, GC-MS generally indicated complete conversion. The ether layer was washed with aqueous citric acid, dried and the compound was purified by MPLC using hexanes/ethylacetate gradient. When the corresponding ketone was the desired product, the crude secondary alcohol was oxidized according to the procedure outlined in Example 3.

EXAMPLE 6

[0045] General procedure for the reductive amination of 2-aryl-3-formyl benzofurans: To a 1 ml vial was added the aldehyde (0.05 – 0.1 mmol), dichloroethane (0.4 ml), 2 equivalents of the primary or secondary amine, 1 equivalent of AcOH and 1.5 equivalents of $NaBH(OAc)_3$. The mixture was stirred vigorously at room temperature. Most reactions were complete in 4-10 hrs as evidenced by GC-MS analysis. The solvent was stripped with a nitrogen stream, ethyl acetate and silicagel (100-200 mg) was added and the crude product was purified by MPLC using the solid sample technique.

EXAMPLE 7

[0046] General procedure for the preparation of 2-aryl-3-aminomethyl benzofurans:

[0047] a) To a dry vial was added triphenylphosphine (275 mg, 1.05 eq.) and phthalimide (149 mg, 1.02 eq.). The vial was stoppered and purged with N_2 . Dry THF (2 ml) was added followed by a solution of the alcohol 2 (as in Formula I, $R^1=CH_2OH$, $R^2=Br$, $X=O$) in THF (1 ml) and immediately by diisopropyl-azodicarboxylate (DIAD), dropwise. The mixture warmed up as the phthalimide dissolved, and the vial was cooled with an air stream. The resulting yellow solution

was stirred at r.t. for 14 hrs. Silicagel was added to the reaction mixture and the solvent was stripped. The desired 2-bromo-3-(phthalimidomethyl) benzofuran was obtained in 84% yield following purification by MPLC using hexanes ethyl acetate 0-40% v/v gradient.

[0048] b) Introduction of various aryl moieties at the 2 position, using the intermediate above, proceeded smoothly following the general Suzuki coupling methodology described in Example 4.

[0049] c) Deprotection of the 2-aryl-3-benzofuranylmethyl phthalimides thus obtained was achieved following treatment of the corresponding intermediate, as a 0.1M solution in isopropanol, with 1.05 equivalents of N,N-dimethyl-1,3-propane diamine and microwave irradiation at 110C for 10 minutes. Upon addition of silicagel the solvent was stripped off and the desired amine was obtained following MPLC using hexanes/ethyl acetate containing 0.1% triethylamine.

EXAMPLE 8

[0050] Intermediates 34b, 35b (amides, ureas, urethanes in the compounds table) were prepared as follows: to a dry vial containing the amines (example NN1, c) (0.1 mmol in 1 ml dry dichloromethane) was added the isocyanate 1.05 eq. and the mixture was stirred at room temperature for 4 hrs or until GC-MS analysis indicated complete conversion. The solvent was stripped, the residue taken in 1 ml ethyl acetate and filtered through a 3 ml SPE cartridge (silicagel) using ethyl acetate. The product was > 95% pure (GC-MS) and was used without further purification. Similarly, acid chlorides were used for the synthesis of amides; in this case, 1 eq. of triethylamine was used as an acid quencher. This procedure was also used for the synthesis of urethanes, when 1.25 eq. of chloroformate was generally needed for the reaction to proceed to completion. Filtration through an SPE cartridge of basic alumina produced compounds of sufficient purity to be used without further MPLC purification.

EXAMPLE 9

[0051] Soluble A-beta (1-42) formation: Soluble A-beta (1-42) (Bachem, Torrance, CA; lot # 0560840 and 0535120) was prepared by first chilling 1 mg of A-beta (1-42) and 375-500 μ L of 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma, St. Louis, MO; HFIP) in separate bottles on ice for 30 min. The cold A-beta (1-42) was solubilized by

adding the cold HFIP and incubating the turbid A-beta -HFIP mixture for 1 hr at room temperature. The resulting clear A-beta -HFIP solution was then dried to a clear film under vacuum. The film was picked up in 22 μ L of dimethyl sulfoxide (Sigma, DMSO) and then diluted with phosphate buffered saline (pH 7.4, PBS) to a volume of 1.107 ml (200 μ M).

[0052] Insoluble A-beta (1-40) fibril formation: A-beta (1-40) fibrils were solubilized in HFIP and then dried to a clear film as described above for soluble A-beta (1-42) formation. The dried clear film of A-beta (1-40) was diluted to 6 mg/mL with filtered distilled, deionized water and incubated, if necessary in a sonic bath, at room temperature until a clear solution was produced. The resulting solution was further diluted with PBS (pH 7.4) to achieve a final concentration of 200 μ M A-beta and then incubated in an orbital shaker at 100-200 rpm for 3-4 days at 37 °C. A cloudy solution was produced, and the fibril concentration was calculated using Congo red. Fibrils were used immediately after their production was confirmed.

[0053] Fluorescence titration assays: Fresh solutions of 2-4 mM probe in methanol were appropriately diluted with PBS (pH 7.4) to obtain assay solutions with final concentration range of 0.1 nM to 100 μ M probe in 300-400 μ L PBS (pH 7.4, 2% methanol) with 10 μ M A-beta (1-40) fibrils or 6.6 μ M soluble A-beta (1-42). A duplicate series of assay solutions were aliquoted with the volume of soluble A-beta or fibrils replaced by PBS. The set of solutions with probe and soluble/fibrillar A-beta and the duplicate set without A-beta were prepared and the fluorescence spectra measured in triplicate. Spectral changes in the probe's fluorescence emission spectra that were unique to the presence of A-beta indicated specific binding interactions between the probe and A-beta species. This unique spectral change allowed for the determination of the probe's binding affinity. Fluorescence emission spectra for binding affinity determinations are measured with fluorometric instrumentation known to those skilled in the art, including at least a spectrofluorometer and spectrograph. Equilibrium dissociation constants were calculated using a one-site saturation model of binding. Similar determinations of equilibrium dissociation constants can utilize instrumentation known to those skilled in the art to measure phenomena including at least absorbance, radioactivity, reflected light, polarized light, mass spectrometry and the like.

EXAMPLE 10

[0054] Select benzofuran congeners exhibited nanomolar binding affinity to soluble A-beta (1-42) but no appreciable binding to A-beta (1-40) fibrils, as determined by fluorescence binding assays. A spectral change of a probe's fluorescence that is unique to the presence of soluble or fibrillar A-beta was used to calculate the binding affinity of a probe. For example, the 22 nM K_D value (Table 3) indicates the selective binding of 37b (Table 2) for soluble A-beta with a characteristic increase in fluorescence intensity (and quantum yield) approaching approximately 5 times the fluorescence intensity (and quantum yield) of 37b in 2% methanol in PBS. 37b in 10 μ M of A-beta (1-40) fibrils had minimal specific binding to the fibrils as demonstrated by an absence of significant enhanced fluorescence intensity in the presence of fibrils. 39b (Table 2) is an example of a benzofuran derivative that specifically binds to A-beta (1-40) fibrils instead of soluble A-beta 42. In constrast, 38b neither binds to A-beta in soluble nor insoluble fibrillar form (Table 3).

Table 3

| Compound | K_D of compound to soluble A-beta (1-42) | K_D of compound to insoluble A-beta (1-40) fibrils |
|-----------------|--|--|
| 38b | Nonspecific binding | Nonspecific binding |
| 39b | No binding < 100 μ M | 1.68 μ M |
| 37b | 22.8 nM | No binding < 100 μ M |

[0055] While the invention has been illustrated and described in typical embodiments, it is not intended to be limited to the details shown, since various modifications and substitutions can be made without departing in any way from the spirit of the present invention. As such, further modifications and equivalents of the invention herein disclosed may occur to persons skilled in the art using no more than routine experimentation, and all such modifications and equivalents are believed to be within the spirit and scope of the invention as defined by the following claims.